YOUNG INVESTIGATORS AWARDS COMPETITION

408 Young Investigators Awards: Physiology, Pharmacology, and Pathology

Monday, March 18, 2002, 11:00 a.m.-12:15 p.m.
Georgia World Congress Center, Room 257W

11:00 a.m.

408-1 Antirestenotic Effects of Inhibition of Balloon Injury Mediated Apoptosis With Local Delivery of a Caspase Inhibitor

Nirat Beohar, James D. Flaherty, Charles J. Davidson, Lee A. MacDonald, Norman C. Wang, Atman P. Shah, Robert Decker, Jon W. Lumasney, Robert O. Bonow, Francis J. Klocke, *Northwestern Univ Medical School, Chicago, Illinois.*

Background: Restenosis is a major limitation of percutaneous coronary interventions. Barotrauma caused by balloon angioplasty has been shown to trigger early onset of apoptosis in vascular smooth muscle cells (SMC), which may promote migration and proliferation. z-VAD.FMK is a broad spectrum synthetic caspase inhibitor that inhibits apoptosis. **Purpose**: To evaluate if a locally delivered caspase inhibitor, z-VAD.FMK, can protect arterial medial SMCs from balloon injury mediated apoptosis, reducing the subsequent SMC proliferation, thereby limiting restenosis.**Methods**: Bilateral iliac artery angioplasty was performed in 12 male NZW rabbits (Acute = 8; Chronic =4). Simultaneous with balloon injury, the artery was treated locally with normal saline (control) or Z-VAD (contralateral artery). Acute animals were treated with high dose (45,000 ng, n=5) or low dose (4,500 ng, n=3) z-VAD and sacrificed at 4 hours. Apoptosis was detected using TUNEL assay. Apoptotic index was calculated (smooth muscle cell nuclei positive for apoptosis/ 200 smooth muscle cells nuclei counted). In chronic studies, high dose (45,000 ng) z-VAD was delivered locally and animals were sacrificed at 4 weeks. Intimal area (Internal elastic lamina area - Luminal area) and medial area (External elastic lamina thickness - Internal elastic lamina area) were measured. **Results**: The reduction in apoptotic index was 45%, ($p<0.001$) with high dose and 33%, ($p<0.02$) with low dose z-VAD. In the chronic animals, the difference in neointimal area was 39% (4.0 ± 0.6 mm2 vs. 2.4 ± 0.5 mm2) ($p=0.0004$) and in medial area was 20% (7.0 ± 0.7mm2 vs. 5.6 ± 0.4 mm2)($p=0.01$) between control and z-VAD treated arteries.**Conclusions**: Significant inhibition of balloon injury mediated apoptosis of arterial SMCs can be achieved using locally delivered z-VAD, resulting in a significant decrease in both neointimal formation and medial proliferation. This novel antirestenotic strategy is in contradistinction to the conventional approach of causing smooth muscle cell death after onset of cell proliferation.

11:15 a.m.

408-2 Adipose Tissue Remodeling Is Coordinated With Vascular Maturation Through Shifts in Angiopoietin-1 Expression

Susan M. Dallabrida, Joseph Upton, David Zurakowski, Judah Folkman, Karen S. Moulton, Maria A. Rupnick, *Children's Hospital, Boston, Massachusetts, Brigham and Women's Hospital, Boston, Massachusetts.*

Background: Adipose tissue is unique in its plasticity and capacity for vascular remodeling. We hypothesized that these characteristics are enabled by specializations in the maturation state and responsiveness of the adipose vasculature. Adipose tissue vessels, if maintained in an immature state, could be more readily mobilized. If so, a shift toward a stable vasculature may be associated with the loss of adipose tissue pliability that occurs in pathologic fat depots.

Methods: Murine epididymal fat from C57BL6/J and ob/ob mice gaining or losing weight was examined for vascular remodeling molecules. Angiopoietin-1 (ang-1), angiopoietin-2 (ang-2), tie-2, and tie-1 RNA (RT-PCR/ Northern) and protein (Western), and tie-2 phosphorylation were measured (immunoprecipitation/ Western). Adipocyte and endothelial cell specific expressions were determined. Normal human adipose tissue was compared to aberrant fat from arterial (AVM), venous (VM), and lymphatic (LM) vascular malformations, macrodactylies, and lipomas.

Results: Adipose tissue growth and regression were associated with decreased ang-1 mRNA, protein, and tie-2 phosphorylation. Ang-2, tie-2, and tie-1 were stable. Ang-1 inversely correlated with absolute rates of weight change, independent of direction (gained, lost) or etiology (TNP-470, leptin, diet). Adipocytes produced ang-1 and endothelial cells expressed ang-2, tie-2, and tie-1. Ang-2 was 76-99 % lower in aberrant adipose tissue, regardless of source (LM, AVM , macrodactylies, lipomas). PECAM mRNA was similar in normal and affected fat indicating that declines in ang-2 were not due to endothelial loss. Ang-1 increased by 44% in lipomas. Tie-2 increased in AVM (41%) and declined in lipomas (47%).

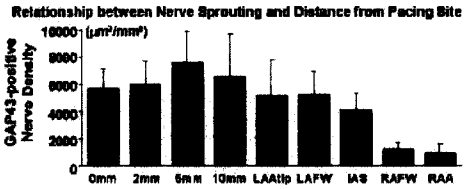
Conclusion: 1) Ang-1 correlates to the rate of change in tissue mass, relating the degree of vessel maturity to the extent of tissue remodeling in normal adipose tissue; 2) Decreased ang-2 in abnormally stable fat suggests a more mature vasculature, which may sustain the affected adipose tissue. Collectively, these findings suggest that specializations in adipose vascular maturation facilitate tissue remodeling, and may offer a point of regulation of adipose tissue mass.

11:30 a.m.

408-3 Induction of Atrial Fibrillation and Nerve Sprouting by Prolonged Left Atrial Pacing in Dogs

Akira Hamabe, Che-Ming Chang, Shengmei Zhou, Yasushi Miyauchi, Yuji Okuyama, Michael C. Fishbein, Hrayr S. Karagueuzian, Lan S. Chen, Peng-Sheng Chen, *Cedars-Sinai Medical Center, Los Angeles, California, UCLA School of Medicine, Los Angeles, California.*

Background: In a canine model of sustained atrial fibrillation (AF) induced by chronic rapid RA pacing, nerve sprouting (NS) is greater in the RA than in the LA. The mechanism is unclear. We hypothesize that NS is induced by electrical current. Therefore, if LA is paced, then NS will be greater in the LA than in the RA **Methods and Results:** Chronic rapid (20 Hz) LA appendage pacing was performed in 5 dogs. Sustained AF (>48 hrs.) was induced within 23±9 days, which was significantly earlier than that with RA pacing using the same protocol (139±84 days). RA, LA and interatrial septum (IAS) were stained with antibodies against growth-associated protein 43 (GAP43) for sprouting nerves and tyrosine hydroxylase (TH) for sympathetic nerves. In all dogs, GAP43-positive-nerve density was highest near the pacing site and decreased with distance from the pacing site. LA and IAS had significantly ($p<0.01$) higher density of GAP43-positive nerves than RA (5723±1579, 4135±1203, 1129±254 mm^2/mm^2, respectively). The TH-positive-nerve density was also highest in LA, followed by IAS and RA (2574±1234, 1487±656, 1007±196 mm^2/mm^2, respectively). The nerves were inhomogeneously distributed within each site, with the greatest heterogeneity observed in LA. **Conclusion:** LA pacing induces sustained AF much faster than RA pacing. In contrast to RA pacing, chronic rapid LA pacing induces greater NS in the LA than in the RA, with the maximum magnitude near the pacing site. These findings indicate that electrical current can induce cardiac NS.



11:45 a.m.

408-4 Acute Neuroprotective Effects of Cortico Steriods Mediated by Nontranscriptional Activation of Endothelial Nitric Oxide Synthase

Florian P. Limbourg, Zhihong Huang, Michael A. Moskowitz, James K. Liao, *Brigham & Women's Hospital, Boston, Massachusetts, Massachusetts General Hospital/Harvard Med. Sch., Boston, Massachusetts.*

Background: Cellular responses to corticosteroids involve the transcriptional modulation of target genes by the glucocorticoid receptor (GR). A rapid, non-transcriptional effect of GR was found to mediate neuroprotection via the activation of endothelial nitric oxide synthase (eNOS).

Methods & Results: In a concentration-dependent manner, dexamethasone stimulated eNOS activity by about 2.5-fold, which was completely inhibited by the GR antagonist, RU486, but not by the transcriptional inhibitor, actinomycin D. Pretreatment with the phosphatidylinositol 3-kinase (PI3K) inhibitor, wortmannin, also inhibited Dex-stimulated eNOS activity, indicating a PI3K-dependent mechanism. Indeed, Dex activated PI3K in a ligand-dependent manner, leading to the phosphorylation and activation of protein kinase Akt and eNOS. In a mouse filament model of transient cerebral ischemia, a bolus injection of Dex (20 mg/kg, i.p.), administered 1 hr before middle cerebral artery occlusion, increased vascular eNOS activity by 2.5-fold, enhanced post-ischemic cerebral blood flow, and decreased cerebral infarct size by 32% (108 ± 9 to 74 ± 8 mm^3, n=11, $P<0.05$). These neuroprotective effects of Dex occurred in the absence of significant changes in physiological parameters and were still evident when Dex was administered 2 hrs *after* ischemia (112 ± 8 to 84 ± 7 mm^3, n=10, $p<0.05$). The beneficial effects of Dex on infarct size were completely absent in $eNOS^{-/-}$ mice, suggesting a novel eNOS-dependent mechanism for stroke protection by corticosteroids.

Conclusion: The non-genomic activation of PI3K/Akt and eNOS by GR represents a physiologically important neuroprotective effect of corticosteroids.

Noon

408-5 The Selective Estrogen Receptor Modulator, Raloxifene, Improves the Severity of Myocardial Ischemia in Canine Hearts

Hisakazu Ogita, Masafumi Kitakaze, Koichi Node, Seiji Takashima, Hiroshi Asanuma, Masanori Asakura, Shoji Sanada, Yoshihiro Asano, Yasunori Shintani, Masatsugu Hori, *Osaka University Graduate School of Medicine, Suita, Japan, National Cardiovascular Center, Suita, Japan.*

Background: We have reported that 17β-estradiol increases coronary blood flow and improves myocardial ischemia. However, little is known as to whether the selective estrogen receptor modulator, raloxifene, mediates coronary vasodilation and improves myocardial ischemia, and what cellular mechanisms are involved in these effects.

Methods: In open-chest anesthetized dogs, the left anterior descending coronary artery (LAD) was perfused through an extracorporeal bypass tube from the left carotid artery. Ral-

oxifene (5μg/kg/min) was infused into LAD through the bypass tube for 20 minutes in either ischemic or nonischemic situations.
Results: In the nonischemic heart, raloxifene had no significant hemodynamic and metabolic effects. However, in the ischemic heart, both coronary blood flow (33±2 to 44±3 ml/100g/min, p<0.01) and fractional shortening (7.9±0.4 to 11.7±0.7 %, p<0.01) were increased by the raloxifene infusion. Lactate extraction ratio (-48.0±4.5 to -16.1±4.8 %, p<0.01) and pH of coronary venous blood (7.271±0.010 to 7.340±0.025, p<0.05) were also increased by raloxifene, indicating the improvement of myocardial anaerobic metabolism. These effects were partially attenuated by pretreatment with either N^G-nitro-L-arginine methyl ester (L-NAME, the inhibitor of NO synthase) or wortmannin (the inhibitor of phosphatidylinositol 3-kinase (PI3-K)), and completely abolished by ICI182780 (the estrogen receptor antagonist) or L-NAME plus charybdotoxin (the blocker of Ca^{2+}-activated K^+ (K_{Ca}) channels). Moreover, increases in both Akt activity and the levels of end products of NO between coronary venous and arterial blood due to raloxifene was completely attenuated by the pretreatment with wortmannin.
Conclusion: These results suggest that both NO and the opening of K_{Ca} channels through the activation of estrogen receptors are mainly involved in the mechanism of improving myocardial ischemia, and that NO production is mediated by PI3-K/Akt pathway.

POSTER SESSION

1122A Assessment and Outcomes of Acute Coronary Syndromes

Monday, March 18, 2002, Noon-2:00 p.m.
Georgia World Congress Center, Hall G
Presentation Hour: 1:00 p.m.-2:00 p.m.

1122A-163 Higher Procedure Use in the United States Versus Canada Among Non-ST Elevation Acute Coronary Syndrome Patients Not Associated With Better Quality of Life Outcomes

Padma Kaul, Yuling Fu, David Knight, Nancy Clapp-Channing, Wanda Sutherland, Christopher Granger, Paul W. Armstrong, Daniel Mark, *Duke Clinical Research Institute, Durham, North Carolina, University of Alberta, Edmonton, Alberta, Canada.*

Comparison of United States (US) and Canadian (CN) practice patterns and outcomes offers a natural experiment to examine the relative benefits of aggressive versus conservative management of non-ST elevation (elev) acute coronary syndrome (ACS) patients (pts) and their differential impact on quality of life (QOL). In a prospectively designed substudy of GUSTO-IIb, we examined QOL at baseline and at one-year among a random sample of 1222 US and 463 CN pts. There was no change in pts' functional status during the 1-year follow-up in either country (Table). By 1-year, CN CABG rates were at par with those in the US. However, CN cardiac catheterization and PCI rates continued to be significantly lower.
In conclusion: contrary to prior findings in the ST-elev MI population, significantly higher procedure rates in the US compared to CN did not translate into improved QOL status in this non-ST-elev ACS cohort. Our QOL results are aligned with those of earlier clinical trials showing no association between aggressive intervention and improved clinical endpoints in this pt population.

Selected QOL Measures & Procedure Rates at Baseline & 1-Year

Description	Baseline		One-Year	
	CN	US	CN	US
Duke Activity Status Index +	20	19	20	19
Current health perception +	73	75	75	75*
Vitality +	50	50	54	50
Mental health +	73	80	87	80*
PROCEDURES (%)				
Cardiac catheterization	45	83*	56	85*
PCI	15	34*	24	39*
CABG	12	19*	24	26

*Inter-country difference was statistically significant at p<0.05 level
+Median values

1122A-164 High-Risk Myocardial Infarction: In-Hospital and One-Year Outcome After Primary Angioplasty and Thrombolysis: A Prospective Nationwide Multicenter Study

Giuseppe Steffenino, Diego Ardissino, Samuele Baldasseroni, Francesco Chiarella, Donata Lucci, Patrizia Maras, Maurizio Marini, Francesco Mauri, Giovanni Maria Santoro, Roberto Violini, Aldo P. Maggioni, on behalf of MISTRAL Investigators, *ANMCO Research Center, Florence, Italy.*

Background. Although primary angioplasty (P-PTCA) is superior to thrombolysis (TT) in randomised trials, this is not true of most registries, and prospective studies in high-risk (HR) pts are scarce. We observed the applicability and the acute and long-term outcomes of either treatment in HR pts with AMI in the community setting.
Methods and Results. At 17 sites with, and 30 without, P-PTCA facilities, 2227 pts with ST elevation AMI 70 y (17.8%), diabetic >70 y (9%), Killip class >1 (23.7%), SBP 100 b/m (2.7%), >4 leads with ST deviation (81.9%), previous MI (11.4%), contraindication to TT (10.2%). P-PTCA was performed in 721 pts, (median door-to-balloon time 50 min), TT in 1090 pts, and in 416 pts (18.7%). No reperfusion treatment (RT) was given. The incidence of the primary in-hospital combined end-point (EP) (death, reinfarction and stroke) was similar in pts with TT (9.2%) and P-PTCA (10.7%) (OR 1.19, 95%CI 0.86-1.63), and it was higher (22.6%) (OR 3.30, 95%CI 2.36-4.63, p<0.001) in pts with noRT. The cumulated 12 months combined EP (death, reinfarction, and new admission for angina or congestive heart failure) was also similar after TT (26.2%) and P-PTCA (24.6%) (OR 0.92, 95%CI 0.74-1.14, p=0.45), and it was higher (45.4%) (OR 2.35, 95%CI 1.86-2.98, p<0.001) after noRT. At multivariate analysis (Cox), age, anterior AMI, killip class <1, heart rate and systolic blood pressure on admission were all significantly associated with both primary EPs. The lack of difference in the outcome of pts after either RT was confirmed by the adjusted analysis. As compared to pts with TT, the subgroup with P-PTCA <60 min from admission showed a trend toward a better in-hospital outcome (OR 0.65, 95%CI 0.42-1.02, p=0.06) and a significant benefit at 1 year (OR 0.69, 95%CI 0.52-0.93, p=0.01).
Conclusions: overall, in this prospective survey, the outcome of pts with HR AMI treated with P-PTCA and TT was similar, with a significant advantage of P-PTCA when performed <1h of admission. Improved management is required for pts currently not receiving reperfusion treatment, whose outcome remains poor.

1122A-165 Six-Year Mortality and Long-Term Risk Model in Older Patients Surviving MI

JoAnne M. Foody, Yun Wang, Martha J. Radford, Harlan M. Krumholz, *Yale University School of Medicine, New Haven, Connecticut, Qualidigm, Middletown, Connecticut.*

Background. Outcomes following myocardial infarction are highly variable and it is often difficult to accurately predict prognosis, particularly in the elderly. Despite the utility of predictive models in clinical practice, few studies have determined the long-term (6 year) prognostic significance of readily available demographic, clinical and functional data in a large national sample of MI survivors.
Methods. We conducted a retrospective cohort study using data from a sample of older patients admitted with AMI in 1992-1993 and a national sample from the Cooperative Cardiovascular Project of patients aged >/= 65 years who survived MI from 1994-1995 to describe the distribution of death after MI and to develop a simple risk model that would accurately stratify elderly survivors of myocardial infarction and predict their long-term (6 year) clinical outcomes.
Results. One year and six year mortality in this cohort of 9118 older patients was 17% and 48% respectively. In the derivation set, the factors with the strongest association with mortality at both 1 and 6 years were older age, female sex, LVEF <40% CHF during admission, diabetes, dementia, prior history of AMI or COPD, length of stay greater than 12 days and renal dysfunction (cr > 2.5). Having a revascularization procedure or cardiac catheterization during the hospitalization was associated with a decrease in mortality. Based on the coefficients in the model, a simple risk score was developed. The C statistic for the derivation model was 0.80 and for the validation model is 0.81.
Conclusions. Mortality rates were high in this older cohort, with the majority of deaths occurring in the first 6 months. We demonstrate that a simple risk model was a powerful tool to risk stratify older patients over the 6 years following AMI and that predictors of risk were remarkably stable over the six years after MI. Knowledge of long-term risk in this elderly cohort of MI survivors will aid in clinical decision making with regard to further diagnostic testing, interventions and secondary prevention strategies.

1122A-166 Diabetes-Related Knowledge Is Not Associated With Measures of Risk Factor Control in Patients With Diabetes and Acute Coronary Syndromes

Carlos D. Sanchez, Darren K. McGuire, L. Kristin Newby, Vic Hasselblad, Mark N. Feinglos, E. Magnus Ohman, *Duke Clinical Research Institute, Durham, North Carolina, Duke University School of Medicine, Durham, North Carolina.*

Background: Diabetic patients have twice the incidence of acute coronary syndromes (ACS) and twice the mortality following ACS compared with non-diabetics. Poor patient understanding of diabetes (DM) is thought to impede appropriate self-management, accelerating cardiovascular complications. We investigated the relationship between patient DM-related knowledge and measures of risk factor control (HbA1c, LDL cholesterol (LDLc), and body mass index (BMI)).
Methods: 200 consecutive patients admitted to a university hospital with DM and ACS (ST-segment elevation and non-ST-segment elevation MI or unstable angina) were enrolled. At enrollment, clinical and demographic data were recorded. Each patient completed a previously validated DM-related knowledge assessment (DRKA). Preliminary results for the first 151 patients are included. Complete results, including 6-month clinical outcomes (death, MI, and revascularization), will be presented.
Results: Median age was 61 years; 38% of patients were female; 62% Caucasian, 27% African-American, and 11% Lumbee Indian. Mean education level completed was 11th grade. Hypertension (86%) and hyperlipidemia (65%) were common; 22% of patients were current smokers. Duration of DM was >10 years in 50% of patients, and 40% were taking insulin. Mean values ± SD were HbA1c 7.9% ± 1.7%; LDLc 105mg/dL ± 36mg/dL; BMI 32kg/m2 ± 8 kg/m2. Years of education and DRKA score were moderately correlated (r=0.501, P<0.0001). HbA1c, LDLc, and BMI showed no correlation with DRKA Score (r=0.009; r=-0.034; r=0.178, respectively). The lack of correlation between DRKA score and risk factor control measures remained after multivariable adjustment for differences in age, race, insulin requirement, duration of DM and years of education.
Conclusion: Among diabetics admitted with ACS, there is moderate correlation between years of education and DM-related knowledge. However, we found no correlation between DM-related knowledge and measures of risk factor control. New strategies must be developed that translate understanding of disease into better risk factor modification among diabetic patients with ischemic heart disease.